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Version 2

Single-molecule Immunofluorescence Tissue Staining Protocol for Oligomer Imaging V.2

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Protocol status: Working

We use this protocol and it's working

Created: January 19, 2023

Last Modified: May 31, 2024

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Keywords: immunofluorescences, oligomer imaging, ASAPCRN, human brain tissue for oligomer imaging, protocol for oligomer imaging, oligomer imaging, molecule immunofluorescence tissue, immunofluorescence, human brain tissue, protocol details background fluorescence quenching, staining protocol, molecule

Abstract

This protocol details background fluorescence quenching and immunofluorescence staining of human brain tissue for oligomer imaging.

Attachments



kb3ib25np.pdf

154KB

Guidelines

- Use only clean bottles, flasks, magnetic stirrers, tweezers, weighing spatulas, measuring cylinders everything should be cleaned, dried and covered if left on the side before next use.
- Everything should be handled with clean tweezers gloves should not touch the samples, solutions and ideally anything placed into the solutions where the slides are.



Materials

Materials and Reagents

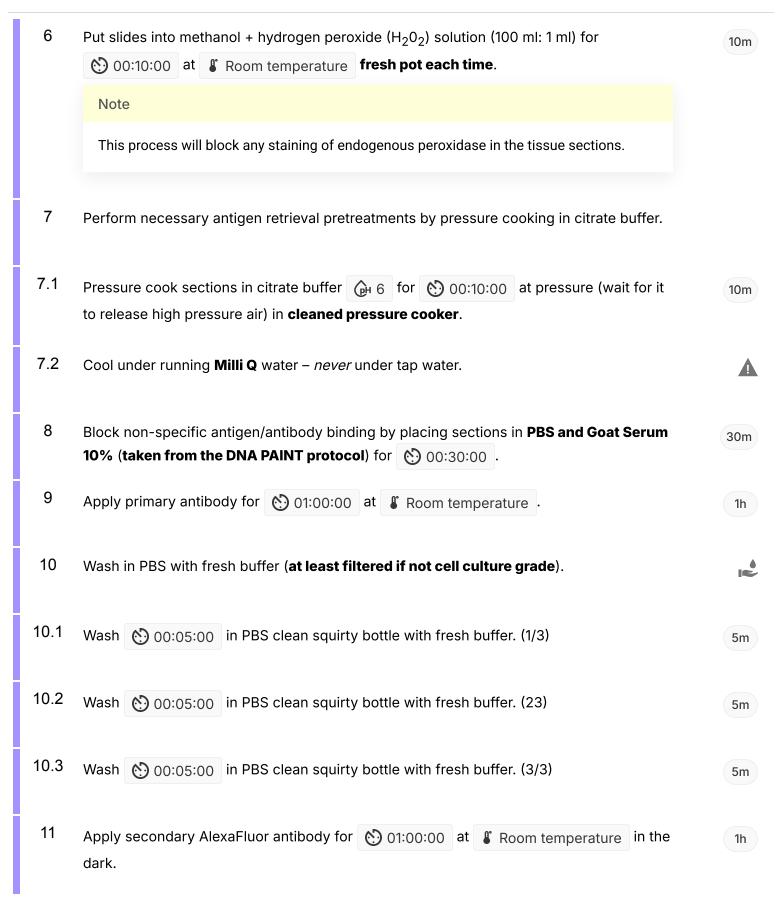
- Microtome
- Glass slides
- Xylene solution
- 100% alcohol
- Methanol
- Hydrogen peroxide (H₂0₂) solution
- Citrate buffer pH6
- Milli Q water
- Pressure cooker
- PBS
- Goat Serum 10%
- AlexaFluor antibody
- 0.1% Sudan black solution
- Vectashield
- Overslip

Troubleshooting



Immunofluorescences staining protocol for oligomer imaging 10m 1 Cut \rightarrow + 8 μ m tissue sections on a microtome and load onto glass slides. 2 Dry slides Overnight at 37 °C - cover over the top. 10m T 3 Before staining commences keep slides for a few hours but ideally Overnight at 10m ₿ 60°C . T 4 De-wax sections through three pots of xylene solution. Use each fresh pots of xylene each time. 4.1 De-wax section through pot of xylene solution for 00:02:00 . (1/3) 2m 4.2 De-wax section through pot of xylene solution for 00:02:00 . (/23) 2m 4.3 De-wax section through pot of xylene solution for 00:02:00 . (3/3) 2m 5 Take sections through two pots of 100% alcohol. Note Use fresh pots each time – methylated spirits. 5.1 Take sections through pot of 100% alcohol for 00:02:00 . (1/2) 2m 5.2 Take sections through pot of 100% alcohol for 00:02:00 . (2/2) 2m







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Take for imaging.

12 Wash in PBS. 12.1 Wash 00:05:00 in PBS in the dark. (1/3) 5m 12.2 Wash 00:05:00 in PBS in the dark. (2/3) 5m 12.3 Wash 00:05:00 in PBS in the dark. (3/3) 5m 13 Add filtered (0.22 um) 0.1% Sudan black solution (0.1% sudan black/70% ethanol) for 10m ⊙ 00:10:00 at \$\mathbb{s}\$ Room temperature in the dark. 14 Wash 2-3 times in 30% ethanol. 15 Mount section with Vectashield and coverslip (Plasma cleaned slides).